Marsupial Spermatogenesis.

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With Plates 15 and 16.

Introduction.

This work is a contribution to the cytology of the Marsupials, a group which, owing to the small number of their chromosomes, affords peculiar advantages over other mammals for this kind of study. The sex chromosomes are particularly clear in this group, and Painter's discovery of a Y-chromosome in the American opossum (Didelphys) has been fully confirmed in several species of Australian Marsupials examined in this laboratory. In the present paper I give the results of the study of three species of Marsupials belonging to two different families, two species belonging to the family Dasyuridae of the sub-order Polyprotodontia, and one to the family Phalangeridae of the sub-order Diprotodontia.

The form examined in most detail is Phascolarctus cinereus. In the other forms I have done little more than determine the number and the behaviour of the sex chromosomes.

The following work was undertaken under the guidance, and with the assistance, of W. E. Agar, F.R.S., Professor of Zoology in the University of Melbourne.

Spermatogenesis of Phascolarctus cinereus.

Material.

The animals were obtained through the courtesy of Mr. Kershaw of the National Museum, from the National Park at

Wilson's Promontory. The first set of testicular and ovarian material was obtained by Professor Agar, who killed and fixed the material. All the other animals obtained were killed and the gonads fixed at the University laboratory. The animals were received at different times during the year, namely in the months of January, April, May, July, and August. In all testis, except those obtained in April, the tubules were filled with various stages in spermatogenesis; in the latter the tubules contained a relatively large number of Sertoli cells, but other stages in spermatogenesis were few in number.

Fixing.

Various methods of fixing the material were employed—Bouin, Allen's modification of Bouin, cold Flemming, and corrosive acetic. The only satisfactory fixatives were the Bouin fluids and the Flemming. For early prophases the Flemming-fixed material gave the best results, but the Bouin fixatives gave very clear figures of the division stages.

Staining.

Sections of 10 μ and 20 μ were cut. Some were mounted in the ordinary way on glass slides, and others were mounted between coverslips so that the nuclei could be examined from both sides.

Heidenhain's iron haematoxylin with iron alum was used for staining the sections. Staining with safranin and gentian violet was also tried, but the results were not very satisfactory.

Number of Chromosomes.

The diploid number of chromosomes in Phascolarctus is sixteen. This number was obtained from numerous counts in both male and female material. Female counts were obtained from the prophases of large cells of the corpus luteum. One such cell is figured (Pl. 15, fig. 1). Altogether sixteen chromosomes can be seen. In the female it should be noted that no chromatic dot is present, such as is shown so clearly in the

spermatogonial metaphase plates (Pl. 15, figs. 2 and 3). In the female there are the comparatively large autosomes (14) and two much smaller chromosomes. These two smaller chromosomes, similar in size and shape, are the sex chromosomes. The chromosome complex of the female is therefore 14+XX.

In the male counts were obtained from equatorial plates of the dividing spermatogonia, and from first and second meiotic division figures. The number of chromosomes obtained from these stages was sixteen. In the spermatogonial plates the chromosomes are arranged in a circle around a central clear space. Inside the circle of chromosomes a chromosome much smaller than the others is seen ; also near this small chromosome a small chromatic dot is to be seen (Pl. 15, figs. 2 and 3). From their subsequent behaviour these are identified as the X- and Y-chromosome respectively. The chromosome formula of the male is therefore 14+XY.

Structure of Testes.

The testes have the typical mammalian structure of numerous convoluted tubules. Close to the wall of the tubules are situated the Sertoli cells, spermatogonia, and the early meiotic prophases. Passing in towards the lumen of the tubule, the later stages of the maturation divisions occur, leading up to the formation of the spermatozoa. These are found nearest the lumen of the tubule. There appears to be a definite layering of the cells of the different stages, although some overlapping of the inner layers occurs.

Spermatogonia.

The nuclei of the early spermatogonia are oval in shape, and are frequently lobed. The nuclei of the later generations of spermatogonia are much smaller and are approximately circular in shape. In the resting condition the spermatogonial nucleus contains a well-defined nucleolus which stains very densely with the iron haematoxylin. This is evidently of the nature of a plasmosome impregnated with chromatin. The chromatin

of the resting cell occurs in the form of rather faintly stained blocks connected by fine strands. The blocks of chromatin appear as loose masses, frayed out at the edges, and the number present is approximately the same as the diploid number of chromosomes.

The onset of the prophase is marked by an increase in the staining capacity of the chromatin blocks which now give rise to short irregular threads. These threads increase in length, probably at the expense of the nucleolus, which now shows up as a large pale body with a few deeply staining granules embedded in it. The long irregular threads begin to contract, ultimately giving rise to the thick chromosomes marking the end of the prophase. The nucleolus has given rise to a large, oval, faintly stained body, a typical plasmosome.

Meiotic Phase.

The origin of the leptotene stage has not been determined with absolute certainty, but it appears that the telophase of the last spermatogonial division does not pass into a complete resting stage, but the chromatin remains in the form of blocks situated close underneath the nuclear membrane. These blocks are present in approximately the diploid number. They are more compact and stain more deeply than the chromatin blocks seen in the resting spermatogonial nucleus. The leptotene stage appears to be derived from this by the formation of long threads from these chromatin blocks. The early leptotene nucleus consists of a tangle of fine threads. On the threads chromomeres can be seen. These are spaced rather far apart and vary considerably in size. In the centre of the nucleus the pale plasmosome can be seen. Following this stage the threads begin to contract away from one side of the nucleus, and, at the same time, begin to contract in length. This is the earliest indication of synizesis (Pl. 15, fig. 4). Although the exact time of syndesis could not be determined, it is probable that it begins to take place now.

It is significant that the leptotene nucleus entering synizesis shows that the threads contract away from that side of the

nucleus which is opposite to that on which the archoplasmic mass is found, and it appears as if this body exerts some influence, if it is not wholly the cause of the synizetic contraction.

In the early leptotene stage no sign of a compact X- or Y-chromosome could be seen, so that they are evidently threaded out like the autosomes at this stage.

Fig. 5 shows a much later stage in syndesis and synizesis. The nucleus is not complete, but shows very clearly the pairing of the chromomeres in homologous threads. The chromomeres exhibit great variability in size. In one of the threads syndesis appears to be nearly complete. In the centre of the nucleus can be seen the compact mass of the synizetic contraction.

The leptotene stage is followed by the pachytene stage. Fig. 6 shows an early pachytene stage consisting of thick, looped chromosomes which have emerged from the synizetic contraction. These chromosomes are seen to be distinctly double in composition, the presence of the chromomeres showing up as darkly stained bodies in the more lightly stained thread. The threads are now very thick. The ends of the threads at this stage are directed towards the archoplasmic mass. Later, these threads lie scattered through the nucleus and all trace of duplicity is eventually lost. The X-chromosome makes its appearance in the early pachytene stage. It appears first as a thin, deeply stained thread (Pl. 15, fig. 7), but contracts down to form a round mass, which is typical of the X-chromosome in later prophase. I have been unable to identify the minute Y-chromosome at this stage. Whether the Y-chromosome fuses with the X-chromosome to form a bivalent could not be determined with certainty owing to the minuteness of the Y-chromosome and the presence in the nucleus at this stage of several deeply staining granules.

Always in contact with the X-chromosome there is a large pale plasmosome (Pl. 15, figs. 7, 8, 9). This varies considerably in shape, and usually contains a number of deeply staining granules. In later pachytene stages two plasmosomes are visible, each containing one or more deeply staining granules

(Pl. 15, fig. 9). One of the plasmosomes (Px) remains in contact with the sex chromosome, the other (Px′) appears to be formed from a division of this plasmosome.

The early pachytene stage is followed by a late pachytene stage in which the chromosomes show a marked diminution in staining capacity. They become diffuse and furry in appearance, the X-chromosome or possible XY bivalent alone remaining as a deeply stained body. The onset of the diplotene stage is marked by the recovery of the staining power of the chromosomes. The chromosomes begin to split so that two long, thin threads are formed. The chromomeres can be distinctly seen on these threads occurring in pairs (Pl. 15, fig. 10).

The thin threads now begin to contract, but never entirely separate from one another, remaining in contact at two or more points (Pl. 15, fig. 11). At this stage the nucleus, which has been increasing in size from the onset of the meiotic prophase, has now attained its maximum volume. Following this stage the nuclear membrane breaks down and the chromosomes lie free in the cytoplasm.

In a few cases during the diplotene stage I have seen the X-chromosome apparently attached to the end of one of the autosomes, but this condition appears to be exceptional. In most cases it lies free. It is in the metaphase of the first meiotic division that the Y-chromosome can first be identified with certainty. Figs. 12 and 13 are of metaphase plates. The first is a cell just after the nuclear membrane has disappeared. The seven large bivalents and the separate X- and Y-chromosomes can be seen attached to one another by threads. Fig. 13 is a rather later view. In Phascolarctus the X- and the Y-chromosome do not usually form a bivalent. In division figures they are always found separate. In the meiotic prophase they may possibly be in the form of a bivalent, but, as mentioned before, this point could not be determined.

Fig. 14 shows a metaphase side-view. In this it will be seen that the X- and Y-chromosomes are on opposite sides of the mass of chromosomes at the equator of the cell, and are travelling to opposite poles of the cell ahead of the other chromosomes.

In many cases, on the other hand, the sex chromosomes lag behind on the spindle. This is shown in fig. 15 of an anaphase. In this figure it will be noted that the X- and the Y-chromosome are attached to the same spindle-fibre.

The first division is therefore the reductional division, and gives rise to two daughter secondary spermatocytes which are dimorphic, one containing the X-chromosome and the other containing the Y-chromosome. This dimorphism of the secondary spermatocytes is shown in fig. 16 (Pl. 16). That these are two daughter spermatocytes is shown by the remains of the spindle-fibres connecting the two cells. In one of the cells can be seen a rather large, deeply stained body in the nucleus which I take to be the X-chromosome. In the other cell nucleus there is a much smaller body not so deeply stained, which probably represents the Y-chromosome.

Second Meiotic Division.

Before the onset of the prophase of the second meiotic division, the secondary spermatocyte undergoes a prolonged resting stage and increases greatly in size. From the resting stage with its faintly staining network, the onset of the prophase is shown by the recovery of the staining power of the chromatin in patches. From this the deeply stained, irregular threads of the prophase are formed (Pl. 16, fig. 17). Right through the meiotic stages the X-chromosome has retained its staining capacity and does not thread out, except in the early prophase of the first meiotic division. During the prophase of the second division the X-chromosome remains compact and does not thread out. The division follows on as before, but this time the sex chromosomes divide, one half going to each pole of the cell. The second division is therefore equational. No further reduction in the number of chromosomes takes place during this division.

Fig. 18 shows an anaphase of the second division with the X-chromosome divided and lagging behind on the spindle. Fig. 19 shows a late anaphase of the same division showing the presence of the Y-chromosome at both poles of the cell.

Cytoplasmic Inclusions.

So far in my description of the spermatogenesis of *Phascolarctus* I have made no mention of cytoplasmic structures. Beyond noting their occurrence in the germ cells I have done little to determine their nature.

In the cytoplasm of the spermatogonia and meiotic stages a large round body is to be seen (Pl. 15, fig. 6). This varies greatly in size and staining capacity at different stages. In some stages it is quite deeply stained, notably in the spermatogonia, leptonema, and early pachynema. Later it stains capriciously, and eventually, during the first meiotic division, becomes quite pale (Pl. 15, figs. 12 and 13). It does not divide during the division but passes indiscriminately to one or other of the secondary spermatocytes (Pl. 16, fig. 16). It occasionally is found in the early spermatid nuclei, but I have been unable to find it at any later stage.

This body, I believe, is probably the same as that figured by Gatenby in his work on the ' Cytoplasmic Inclusions of Germ Cells ', as an excretory granule.

Another cytoplasmic inclusion seen in all stages of spermatogenesis is a very pale, somewhat sausage-shaped body lying close up against the nuclear membrane (Pl. 15, figs. 6 and 10). It is towards this body that the synizetic contraction takes place. It is found in all the secondary spermatocytes and spermatids, although I have never found any sign of its division during any of the stages in spermatogenesis. From its behaviour I believe this to be the archoplasmic mass.

Other cytoplasmic inclusions are the chromatoid bodies. These are conspicuous in the sections fixed with Flemming, but are pale and inconspicuous in those sections fixed in Bouin.

The leptotene nucleus always contains one or more deeply staining granules. In the pachytene stage also the nucleus often contains a deeply stained granule usually lying close beneath the nuclear membrane (Pl. 15, fig. 7). These granules appear to give rise to the chromatoid bodies seen in the cytoplasm of the germ cells at different stages (Pl. 16, fig. 28).

These chromatoid bodies vary in size and in number. During the first meiotic division they are distributed indiscriminately between the two daughter cells. Further than this they have not been traced.

The Sertoli Cell in Phascolarctus.

In Phascolarctus the Sertoli cell nucleus is very large (Pl. 16, fig. 20). It is about three times as large as the nucleus of the primary spermatocyte when it has attained its maximum volume. The nucleus lies at the foot of the Sertoli cell close to the wall of the tubule. The outlines of the cell could not be distinguished. The chromatin of the nucleus is in the form of a coagulum distributed through the nucleus. In the centre of the nucleus there is a clear space surrounding a granular mass. This granular mass is probably formed by the degeneration of the nucleolus.

The cytoplasm of the Sertoli cell contains, usually lying close up against the nucleus, a varying number of refractive, rod-like bodies. These are a constant feature of the Sertoli cell in Phascolarctus, and I found them present in all the material examined by me whatever time of the year the material was obtained. Usually in close association with the rods, a pale yellow, fluffy mass can be seen. This probably consists of deutoplasmic material. This shows up well in sections which have been fixed in cold Flemming before staining with the iron haematoxylin.

In some of the outside tubules of the sections, especially in the Flemming-fixed material, instead of the bundle of rods a mass of over-lapping, very pale plates can be seen (Pl. 16, fig. 21).

The rods appear pale yellow in those sections fixed in the Bouin, but often in the Flemming-fixed material are quite black. The rods are found together, lying approximately parallel. They vary in length, some of them consisting of small pieces lying end to end and probably formed by the fracture of one of the longer rods. Although the rods are usually comparatively straight, in many cases they are seen to possess a very wavy outline.

Much has already been written regarding these rod-like bodies present in the cytoplasm of the Sertoli cells in Phascolarctus. I do not propose to discuss at much length the theories already advanced, but will give my conclusions arrived at by a study of these bodies, and the results of experiments undertaken by me to determine if possible their nature. The experiments were undertaken with the view to ascertaining whether the rods served a nutritive function. The fact that these rods were found only in the Sertoli cells led me to believe that possibly these rods were a source of nutriment, or connected in some way with the supply of nutriment to the developing spermatozoa.

Below I give the results of some digestion experiments undertaken to prove this point if possible.

Fresh material was cut by means of a freezing microtome. Owing to the difficulty of picking up the rods in unstained material, the tissue was stained. The stains used were neutral red and methyl green.

The sections after staining were submitted to the action of a weakly acidic mixture of pepsin and glycerine. Cells with rods were picked out and their position marked by means of a micrometer eyepiece. Then the microscope was placed in an electric oven and kept at a constant temperature of 30° Centigrade. The progress of the action was watched from time to time. The accumulation of the products of digestion after a time tended to stop the action and only partial digestion took place. In all cases of partial digestion the rods were still visible. With the use of more of the digestive fluid complete digestion of the cytoplasm occurred. In this case, with complete digestion, the cells moved about in the fluid and were difficult to find again. In the majority of the experiments the rods were identified even after complete digestion, but in some cases they could not be found.

The same experiment was carried out, using an alkaline mixture of zymine and glycerine. Again, here the rods were still visible after partial digestion ; but in the case of complete digestion, owing to the difficulty of picking up the rods in the

resulting fluid, I could not be absolutely certain whether the rods were dissolved or not, although in the majority of cases the rods were still visible.

Bardeleben refers to these bodies as crystals of haematoidin. He also describes the presence of similar bodies in the great blood lacunae in the material of the testis. With regard to the composition of these bodies I have not been able to determine whether they are composed of haematoidin or not.

Fresh tissue was boiled in chloroform. The tissue was then embedded and sections cut, stained, and mounted. Upon examination it was found that in no case did the chloroform have any action on the rods in the Sertoli cells.

This does not of course confirm Bardeleben's view that they are crystals of haematoidin, but goes to show that they are, at any rate, not a blood derivative which is an acid.

With regard to the occurrence of similar bodies in the blood lacunae of the testis, in all the material I have examined I have not found any bodies comparable with the rods found in the Sertoli cells. The presence of somewhat similar rods has been described by several authors. Montgomery has shown that the Sertoli cells in man are derived from the spermatogonia, and that the nature of the resultant cell is determined by the presence of a rod-like body in the cytoplasm, i. e. all spermatogonial cells containing the rod-like body give rise to the Sertoli cells. This, however, does not appear to be the case in Phascolarctus. Although I am convinced that the Sertoli cells in Phascolarctus arise from a division of the spermatogonia, I have never been able to find any trace of the rods until the nucleus of the cell, by its peculiar structure, is definitely defined as a Sertoli cell. No Sertoli cell divisions have been found in Phascolarctus, but in Perameles (in this animal the Sertoli cells are comparable with Phascolarctus in point of size and nuclear structure) I have found an apparent diplotene nucleus which from its size and position in the tubule appears to have originated from a Sertoli nucleus. From this it seems safe to assume that the Sertoli cell has been derived from the same cells as give rise to the germ cells.

Spermatogenesis of Sarcophilus ursinus.

Both male and female animals of this species were obtained. The technique followed was the same as in Phascolarctus, viz. fixatives used were Allen's modification of Bouin, and cold Flemming; this was followed by staining in Heidenhain's iron alum haematoxylin. In the testis the same more or less definite layering of the germ cells in the tubules is noted as in Phascolarctus.

Number of Chromosomes.

In the female the number of chromosomes was obtained from metaphase plates of dividing follicle cells surrounding the ovum. The number found was fourteen. Of this number twelve are large and the other two are much smaller. These two smaller ones, similar in size and shape, are the sex chromosomes. The chromosome formula of the female Sarcophilus is therefore 12 + XX (Pl. 16, fig. 22).

In the male chromosome counts were obtained from spermatogonial plates and first meiotic division stages.

From the metaphase plates of the spermatogonial divisions the number of chromosomes was found to be fourteen. Here, as in Phascolarctus, the presence of a small X-chromosome and a minute Y-chromosome, usually in the centre of a circle formed by the twelve larger autosomes, was again noticed (Pl. 16, fig. 23). In fig. 24 the spermatogonial chromosomes are dividing or have already divided. Two of the chromosomes as yet show no sign of splitting (Pl. 16, fig. 24, *a* and *b*). The division of the Y-chromosome is very clearly shown in this figure.

The chromosome formula of the male Sarcophilus is therefore 12 + XY.

Meiotic Phase.

I have made no attempt to follow out in detail the phenomena of the meiotic phase, but stages similar in appearance to those of Phascolarctus are found in Sarcophilus.

The side-view of an early anaphase of the first meiotic division is shown in fig. 25. The X- and the minute Y-chromosome are on opposite sides of the central mass of chromosomes, and are travelling towards the poles of the cell ahead of the other chromosomes. The first meiotic division therefore acts as the reductional division and the two daughter secondary spermatocytes produced are dimorphic, one containing the X-chromosome and the other containing the Y-chromosome. Satisfactory second meiotic division figures have up to the present not been obtained.

Spermatogenesis of Dasyurus maculatus.

Only the male of this species was obtained, and so a check count of the number of chromosomes of the female could not be obtained. However, very good counts from spermatogonial metaphase plates were obtained leaving no doubt as to the number of chromosomes present. The number of chromosomes in this animal is the same as in Sarcophilus, a member of the same family. Of the fourteen chromosomes, twelve are the autosomes, one is the small X-chromosome, and the other the minute Y-chromosome (Pl. 16, figs. 26 and 27).

From figures of the first meiotic division, the separation of the X- and the Y-chromosome is seen to take place as in the other animals (Pl. 16, fig. 28).

Summary.

In the three animals studied the total number of chromosomes in the male is as follows :

Phascolarctus	16 (14 autosomes + XY).
Sarcophilus	14 (12 autosomes + XY).
Dasyurus	14 (12 autosomes + XY).

In the female the number of chromosomes is as follows :

Phascolarctus	16 (14 autosomes + XX).
Sarcophilus	14 (12 autosomes + XX).

In all animals dealt with in this paper the Y-chromosome is very minute in size compared with the other chromosomes ;

also the X-chromosome is much smaller than any of the autosomes.

Chromomeres are conspicuous during syndesis, early pachytene, and early diplotene stages.

The early pachytene stage is followed by a late pachytene stage in which the threads become diffuse and lose their capacity for taking up the stain.

Except in the early meiotic prophase the sex chromosome remains compact and deeply stained and does not thread out like the autosomes.

In all the above animals the first meiotic division is reductional, separating the X- and the Y-chromosomes, and the second division is equational, in each cell the sex chromosome dividing. The spermatozoa are therefore of two kinds, one containing an X-chromosome and the other containing a Y-chromosome.

No further reduction in the number of chromosomes takes place during the second meiotic division.

The Y-chromosome could not be identified during the meiotic phase until the metaphase of the first meiotic division. At this stage in Phascolarctus the sex chromosomes are separate and do not form a bivalent.

The archoplasm seems to exert some influence on the chromatin threads at synizesis and during the early pachytene stage. In the former case the contraction takes place to that side of the nucleus at which the archoplasmic mass is situated ; in the latter the chromosomes are in the form of thick loops with the ends of the chromosomes pointing towards the archoplasmic mass.

In Phascolarctus the Sertoli cells are very large and possess peculiar rod-like bodies, the origin and function of which was not arrived at. The result of experiments seem to show that the rods are not affected by the action of digestive fluids.

EXPLANATION OF PLATES 15 AND 16.

Reference Letters.

A, archoplasmic mass. *E*, excretory body. *D*, deutoplasm. *P*, plasmosome. *Px*, Plasmosome associated with X-chromosome. *Px'*, plasmosome derived from *Px*. *Chr*, chromatoid body.

Plate 15.

Phascolarctus cinereus.

Fig. 1.—Female. Nucleus of a cell from the corpus luteum. The two sex chromosomes (XX) are much smaller than any of the fourteen autosomes. (Bouin.)

Fig. 2.—Spermatogonial plate with fourteen autosomes surrounding the X- and Y-chromosomes. (Bouin.)

Fig. 3.—Spermatogonial plate. The constrictions seen at the end of some of the chromosomes are not usually present. (Bouin.)

Fig. 4.—Late leptonema, and beginning of synizesis. Only some of the threads are shown. Chromomeres are distinct on some of the threads. (Flemming.)

Fig. 5.—Late synizesis and syndesis. Incomplete nucleus. Showing the pairing of the chromomeres on homologous chromosomes. Central mass representing the synizetic contraction. (Bouin.)

Fig. 6.—Early pachynema. Thick-looped chromosomes still showing some duplicity. (Flemming.)

Fig. 7.—Showing the condensation of the sex chromosome, also the associated plasmosome containing deeply staining granules. *C*, chromatic granule in nucleus which probably gives rise to chromatoid body. (Bouin.)

Figs. 8, 9.—Showing the presence of the second plasmosome (*Px'*). (Bouin.)

Fig. 10.—Early diplonema. Compact X-chromosome or XY bivalent. Paired chromomeres distinct.

Fig. 11.—Later diplonema. (Bouin.)

Fig. 12.—Metaphase plate of first meiotic division just after nuclear membrane has disappeared. (Bouin.)

Fig. 13.—Later metaphase plate. (Flemming.)

Fig. 14.—Metaphase (side-view). X- and Y-chromosomes travelling to opposite poles of the cell ahead of the other chromosomes. (Bouin.)

Fig. 15.—Anaphase of first meiotic division. X and Y lagging behind on the spindle. (Bouin.)

Plate 16.

Phascolarctus cinereus.

Fig. 16.—Daughter secondary spermatocytes (dimorphic) with remnants of spindle-fibres between the two cells. One cell nucleus contains a deeply stained body—the X-chromosome. (Bouin.)

Fig. 17.—Prophase of second meiotic division. Irregular threads. X-chromosome compact. (Flemming.)

Fig. 18.—Anaphase of second meiotic division showing division of the X-chromosome. (Bouin.)

Fig. 19.—Anaphase of the second meiotic division showing division of the Y-chromosome. (Bouin.)

Fig. 20.—Sertoli cell nucleus with associated rods and deutoplasm. (Flemming.)

Fig. 21.—Sertoli cell nucleus with accompanying pale plates. The chromatin of the nucleus is represented semi-diagrammatically. (Flemming.)

Sarcophilus ursinus.

Fig. 22.—Female. Metaphase plate of follicle cell. 12+XX chromosomes. (Flemming.)

Fig. 23.—Spermatogonial plate. 12+XY chromosomes. (Bouin.)

Fig. 24.—Splitting of chromosomes during spermatogonial mitosis. All chromosomes except two (*a* and *b*) have divided. (Bouin.)

Fig. 25.—Metaphase (side-view) of first meiotic division showing separation of the X- and the Y-chromosomes. (Bouin.)

Dasyurus maculatus.

Fig. 26.—Spermatogonial plate. 12+XY chromosomes. (Bouin.)

Fig. 27.—Spermatogonial plate. 12+XY chromosomes. (Bouin.)

Fig. 28.—Metaphase (side-view) of first meiotic division showing the separation of the X- and the Y-chromosomes. (Flemming.)

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